

Institut für Parasitologie  
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med. vet. Peter Deplazes

Arbeit unter der wissenschaftlichen Betreuung von  
Prof. Dr. med. vet. Manuela Schnyder

**Land-use and altitude-dependent prevalence rates of *Angiostrongylus vasorum* and other  
parasites of red foxes (*Vulpes vulpes*)**

**Inaugural-Dissertation**

zur Erlangung der Doktorwürde der  
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

**Barbara Koller**

Tierärztin

von Rünenberg, BL

genehmigt auf Antrag von

Prof. Dr. med. vet. Manuela Schnyder, Referentin

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## Zusammenfassung

In Anbetracht der Rolle des Fuchses als Reservoir für *Angiostrongylus vasorum*, einem Herz-Lungen-Nematoden von Hunden und Füchsen, wurde dessen Vorkommen in Schweizer Füchsen mittels einer standardisierten Methode untersucht. In 72 Gebieten, bestehend aus je drei Quadratkilometern, wurden durch Ablaufen von linearen Transekten im Terrain 1481 Fuchskot-Proben gesammelt und mittels einer Flotations-Sieb-Methode analysiert. Die Prävalenz von *A. vasorum* war 8.8% (95% Konfidenz-Intervall, CI: 7.4-10.3%), wobei sie im Mittelland (11.4%, CI: 9.4-13.8%) und in Gebieten mit weniger als 50% Landwirtschaftsfläche höher war ( $p=0.043$ ). Die Prävalenzen nahmen mit zunehmender Höhe ab und waren höher in Gebieten unterhalb von 400 m ü. M. (20.6%, 95% CI: 15.2-26.9%), während alle Proben über 900 m ü. M. negativ waren. Ausserdem wurden Eier von *Toxocara* sp. (12.1%), Taeniidae (10.5%), *Capillaria* spp. (8.3%), *Trichuris vulpis* (5.5%), Hakenwürmern (5.3%), *Toxascaris* (1.3%) und *Strongyloides* spp. (0.4%) identifiziert. Taeniiden-Eier waren positiv und *Capillaria* spp. negativ assoziiert mit dem Umfang an bewirtschaftetem Land. Die auf einem Koordinaten-Netz basierende Fuchskot-Sammlung mit reproduzierbaren Transekten erwies sich als eine geeignete, nicht-invasive Methode für eine umfassende parasitologische Überwachung, welche Informationen über den relativen Infektionsdruck auf die lokale Hundepopulation liefert.

Schlüsselwörter: Helminthen, Standardisierung, Fuchskot, Bioregionen, Schweiz

## Summary

In view of the role of foxes as a reservoir for *Angiostrongylus vasorum*, a cardiopulmonary nematode of dogs and foxes, its occurrence across Switzerland was examined by using a standardised sampling scheme for fox faecal samples. In 72 study areas consisting of three one square km grid cells, a total of 1481 samples were collected by walking transects following linear features in the terrain and analysed by a flotation-sieving method. The overall prevalence rate of *A. vasorum* in faecal samples was 8.8% (95% Confidence Interval, CI: 7.4-10.3%), being significantly higher in the Swiss Plateau (11.4%, CI: 9.4-13.8%) and in areas with less than 50% of cultivated land ( $p=0.043$ ). Prevalence rates decreased with increasing altitudes being significantly higher below 400 m above sea level (20.6%, 95% CI: 15.2-26.9%), while all samples collected above 900 m asl were negative. Eggs of *Toxocara* sp. (12.1%), Taeniidae (10.5%), *Capillaria* spp. (8.3%), *Trichuris vulpis* (5.5%), hookworms (5.3%), *Toxascaris* (1.3%), *Strongyloides* spp. (0.4%) were also identified. Taeniid eggs were positively and *Capillaria* spp. negatively associated with the portion of cultivated land. The grid-cell based sampling scheme with replicable transects for fox faeces collection proved to be a suitable, non-invasive method for large-scale parasite surveys providing information on the relative infectious pressure for the local dog population.

Key words: helminths, standardisation, fox faeces, bioregions, Switzerland

# **Land-use- and altitude-dependent prevalence rates of *Angiostrongylus vasorum* and other parasites of red foxes (*Vulpes vulpes*)**

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## **Abstract**

In view of the role of foxes as a reservoir for *Angiostrongylus vasorum*, a nematode of the heart and lungs of dogs and foxes, its occurrence across Switzerland was investigated in foxes applying a standardized sampling scheme for fox faecal samples. In 72 study areas, which consisted of three one square km grid cells, a total of 1481 samples were collected by walking transects following linear features in the terrain and analysed by a flotation-sieving method. The overall prevalence rate of *A. vasorum* in faecal samples was 8.8% (95% Confidence Interval, CI: 7.4-10.3%), being significantly higher in the Swiss Plateau (11.4%, CI: 9.4-13.8%) compared to other bioregions, and more prevalent in areas with less than 50% of cultivated land ( $p=0.043$ ). Prevalence rates increased with decreasing altitudes being significantly higher below 400 m above sea level (20.6%, 95% CI: 15.2-26.9%), while all samples collected above 900 m asl were negative. Eggs of *Toxocara* sp. (12.1%), Taeniidae (10.5%), *Capillaria* spp. (8.3%), *Trichuris vulpis* (5.5%), hookworms (5.3%), *Toxascaris leonina* (1.3%) and *Strongyloides* sp. (0.4%) were furthermore identified. Taeniid eggs were positively and *Capillaria* spp. negatively associated with the amount of cultivated land. The prevalence rates based on our faecal analyses were generally lower compared to previous studies from Switzerland which were based on fox necropsies. However, the grid-cell based sampling scheme with replicable transects for fox faeces collection proved to be a suitable, non-invasive method for parasitic large-scale surveys providing information on the relative infectious pressure for the local dog population.

**Key words:** helminths, standardized method, fox faeces, epidemiology, bioregions, Switzerland

## Introduction

*Angiostrongylus vasorum* is a 13- 21 mm long metastrongylid nematode, which mainly affects foxes and dogs, but also wolves, badgers and other wild carnivores. The parasite is transmitted by ingestion of snails of different species (Guilhon and Bressou 1960). In its definitive host, *A. vasorum* resides in the pulmonary arteries and the right side of the heart. The parasite causes cardio-respiratory and neurological signs as well as bleeding disorders especially in dogs, with a potentially fatal outcome (Chapman et al. 2004; Koch and Willesen 2009; Staebler et al. 2005).

Traditionally, *A. vasorum* occurred in a patchy pattern with endemic foci in southern France (Cuillé and Darraspen 1930; Guilhon 1963) and Ireland (Dodd 1973; Roche and Kelliher 1968), followed by reports from Denmark (Bolt et al. 1992). During the last decades findings from other regions in Great Britain (Helm et al. 2015; Morgan et al. 2008; Schnyder et al. 2013a), on the Iberian peninsula (Alho et al. 2014; Gerrikagoitia et al. 2010; Segovia et al. 2004) and in central (Barutzki and Schaper 2009; Bolt et al. 1992; Jolly et al. 2015; Staebler et al. 2005; Taubert et al. 2008; van Doorn et al. 2009), southern (Guardone et al. 2013; Magi et al. 2009; Papazahariadou et al. 2007; Tieri et al. 2011), northern (Grandi et al., 2017) and eastern Europe (Hurnikova et al. 2013; Majoros et al. 2010; Rajkovic-Janje et al. 2002; Schnyder et al. 2013b) indicate that this parasite becomes more and more important, occurring in areas which were not reported as endemic so far.

Several factors influencing the distribution pattern of *A. vasorum* and the apparently increasing number of reported dogs suffering from the disease are under discussion. Travelling with untreated dogs from endemic to non-endemic areas could play a role for the expansion of *A. vasorum* into distant, previously parasite-free, areas. In contrast, climatic factors, such as mean winter temperatures below -4 ° C may represent a barrier for the establishment of *A. vasorum* (Jeffery et al. 2004; Lurati et al. 2015). Importantly, in areas with a dense fox population, foxes may contribute to the endemic establishment and an increased parasitic infection threat, representing a reservoir and therefore a risk for the dog population. Foxes are well adapted to urban habitats and considered as opportunists concerning their nourishment, and urban areas offer them favorable terms for associations to family groups and to live in high density populations (Gloor et al. 2001). This fact is of concern because foxes and dogs are sharing most of their parasites: *Toxocara canis*, *Capillaria* spp., *Uncinaria stenocephala*, *Taenia* and *Echinococcus* spp., *Mesocostoides* spp., *Dipylidium caninum* (Fischer et al. 2005; Hofer et al. 2000; Willingham et al. 1996), and *A. vasorum* and *Crenosoma vulpis* as well.

In Switzerland, first cases of *A. vasorum* were reported from a dog breeding station in Zurich in 1968 (Wolff et al. 1969), but only approximately 30 years later Staebler et al. (2005) reported about five dogs infected with *A. vasorum* in the northern part of Switzerland, and about 3 dogs coming from southern Ticino, all diagnosed between 1999 and 2004. In 2001, two infected foxes originating from the region of Basel were reported (Gottstein 2001). Since 2004 up to now, numerous additional foxes and new cases of canine angiostrongylosis have been identified (Gillis-Germitsch et al. 2017; Sigrist et al. 2017), partly in the same regions as already reported, but also in new areas, as for example in the eastern and French part of Switzerland (Lurati et al. 2015). In dogs, definitive diagnosis is typically attained by isolation of first stage larvae (L1) from faeces through larval migration adopting the Baermann-Wetzel technique (Deplazes et al. 2016) or by serological methods (Schnyder et al. 2014; Schnyder et al. 2011). In contrast, prevalence data on foxes mostly rely on culled animals and correlated necropsy findings and/or adopting serological methods adapted to foxes (Gillis-Germitsch et al. 2017; Houpin et al. 2016), therefore implying selection based on killed animals.

In view of the role of foxes as a reservoir for *A. vasorum* and of the increasing number of reports of canine angiostrongylosis, epidemiological investigations on foxes were performed applying a standardized sampling scheme based on the collection of fox faecal samples along transects in 1-km<sup>2</sup> grid cells dispersed over the whole country, and adopting copromicroscopic analyses



allowing the detection of endoparasites. The aim of this study was to investigate the large-scale distribution of *A. vasorum* and other helminths in the Swiss fox population, to identify environmental factors enhancing their presence and therewith to assess the threat of infection for the Swiss dog population.

## Materials and Methods

### Research area and sampling methods

Fox faeces were collected in 72 localities distributed across Switzerland (total area: 41'285 km<sup>2</sup>). We selected 54 localities in the low lands of Switzerland (< 700m asl) and 18 in higher altitudes (range 700-1'800m, mean 929m, median 800m). A total of 58 localities were situated in the North and 14 in the South of the Alpine crest. In all but one locality, where for topographic reasons only one plot could be selected, three sampling plots were determined. Each sample plot of such a triplet corresponded to a grid cell of the Swiss coordinate system which is based on 1-km<sup>2</sup> grid cells (<http://map.geo.admin.ch>). Each of the three grid cells of a triplet was situated in a distance of 1 km from the next grid cell. This sampling scheme was implemented in order to obtain samples from different fox families at each locality, as typical fox home range sizes vary between 20-30 ha in urban areas (Gloor et al. 2001) and are generally smaller than 1 km<sup>2</sup> for reproductive individuals in landscape with high food resources (Gloor et al. 2001; Henry et al. 2005). The plots were chosen to include agricultural area (cultivated land), forest and human settlements (at least 5% of each category). Fox faeces were collected in nine localities between August 2010 and February 2011, in 51 between June 2011 and January 2012 and in twelve localities during both periods (data were pooled for the statistical analyses).

Based on methods in use for monitoring changes in the fox population density (Webbon et al., 2004), a modified technique for a standardized collection of faecal samples was developed. In detail, tracks of 5 to 6 km per each km<sup>2</sup> were followed using GPS (Garmin, GPSMAP® 60CSx) and the exact positions of faecal samples were logged. The scats were judged concerning their odour, the spot where they were discovered (i.e. in clearly visible and, possibly, elevated spots, characteristic of foxes for territorial marking; or near a cluster of rodents or near carrion indicating successful food recovery), and with details about their content (fruits, pits, bones, hair) based on operating experience and internal documentation to confirm identity and exclude non-fox faeces. Samples with no clear characteristics of fox scats were not collected.

### Flotation-sieving method and morphological identification

In order to minimize health risks due the potential presence of *E. multilocularis* eggs in fox faeces, the collected samples were initially stored at -80 °C for three days, afterwards at -20 °C until examination. Faecal flotation was performed as previously described for the isolation of eggs of *E. multilocularis* (Deplazes and Eckert 1996), with modifications. First, the samples were centrifuged at 1600g for 10 min, the supernatant was discarded and the sediment mixed with 8 ml of zinc chloride (specific gravity: 1.45). After vortexing, the samples were centrifuged at 1000g for 30 min, followed by two sieving steps: after pouring the supernatant through a 150-µm mesh sieve, the sediment was poured through a 21-µm mesh sieve, in which L1 were supposed to accumulate. Therefore, the material retained in the sieve was washed out with water and poured into a 3.5 ml petri dish. Parasitic stages were identified based on morphological structures (Deplazes et al., 2016) using an inverted light microscopy by a 320-fold magnification (Labovert FS, Leitz Wetzlar, Germany).

### Molecular identification

A part (69/115) of the samples in which L1 of *A. vasorum* were morphologically identified were verified by a specific *A. vasorum* - PCR targeting a region of the second internal transcriber spacer (ITS-2) of *A. vasorum* (Jefferies et al. 2009) after DNA-isolation (Stefanic et al. 2004). Also from 110 samples with morphologically unidentified larvae, from 11 out of 317 samples containing morphologically identified L1 of *C. vulpis*, from one sample containing L1 of *C.*

*vulpis* and contemporaneously non-parasitic soil nematode larvae, and from 18 samples containing soil nematode larvae only the same PCR was performed. In detail, the larvae were transferred into tubes and centrifuged for 1 min at 13'000 rpm. The supernatant was discarded and the residue was mixed with 200 µl of TE buffer (1 ml 1M Tris- HCl pH 8.4, 0.2 ml 0.5 M EDTA pH 8.0, 98.8 ml aqua dest.) and swung twice with a swing mill (model: MM 300, Retsch GmbH, 42781 Haan, Germany) at 30 1/s for 1 min using 3 mm stainless steel balls (Schieritz & Hauenstein AG). Between and after these two swing steps, the samples were promptly centrifuged and cooled down. After incubating at 95 °C for 5 min, the samples were again cooled down, followed by centrifugation at 13'000 rpm for 1 min. Afterwards, 20 µl protein kinase K and 200 µl of buffer AL (guanidine hydrochloride) were added, using a commercial kit for DNA purification (Qiaamp DNA mini kit®, Qiagen, Hilden, Germany). The samples were incubated at 56 °C for 4 hours, followed by step 5 of the manufacturer's protocol for purification from blood or body fluid, with a final elution volume of 200 µl. Conventional PCR assays were performed in a final reaction volume of 100 µl. Primers and conditions were used as described by Jefferies et al. (2009) with some adaptations: 35 cycles were performed in a Peltier thermal cycler (PTC-200, Bio- Rad Laboratories AG, Reinach, Switzerland). The final extension step at 72 °C lasted 10 minutes. A negative control (no DNA) and a positive control, consisting of 2 µl PCR confirmed *A. vasorum*- DNA and 8 µl of water, were included in all tests. Amplicons were detected on 1.5% agarose gels stained with Gel Red (Biotium, Chemie Brunschwig AG, Basel, Switzerland).

Thirteen samples in which the PCR results were not congruent with the microscopic findings were tested for the purpose of discovering an inhibition of the amplification reactions as described by Mathis et al. (1996), and a validation was done by a second PCR using the nematode primers NC1 and NC2, also targeting a part of ITS-2, as previously described (Romstad et al., 1997). The samples (n=5) in which larvae were morphologically determined as L1 of *A. vasorum*, being negative in the specific *A. vasorum* – PCR but positive in the broader PCR for nematodes were sequenced. For this purpose, the amplicons detected on 1.5% agarose gels as was excised and extracted following the protocol (MinElute handbook of Qiagen), the concentration measured by spectrophotometry (NanoDrop® ND-1000 spectrometer, Thermo Scientific) and submitted for sequencing (Synergene, Switzerland).

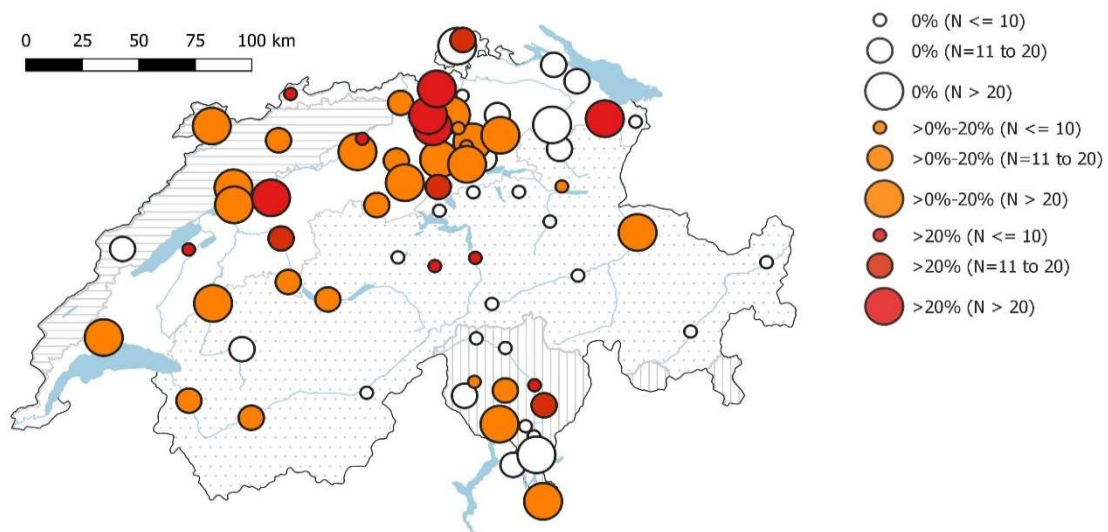
### Spatial and statistical analysis

QuantumGIS version 1.8.0 (<http://qgis.org/>) was used to build maps and to characterize the 72 study areas in regard on the land use in these regions. Based on the Swiss Federal area statistics 2004/09, which differentiates 74 land use categories based on a one-hectare grid, we calculated for each study area the percentage of urban and cultivated area (urbanized area: categories 20-68, cultivated land: categories 71-89, see Arealstatistik Schweiz on [www.bfs.admin.ch](http://www.bfs.admin.ch)). We further attributed to each study area a bioregion (Alps [including Northern, Western Central and Eastern Central Alps], Central Plateau, Jura and Southern Alps) corresponding to the biogeographic regions set by Gonseth et al. (2001) and used the mean number of faecal samples found per km<sup>2</sup> (FAECES FREQUENCY) as rough indicator of fox density.

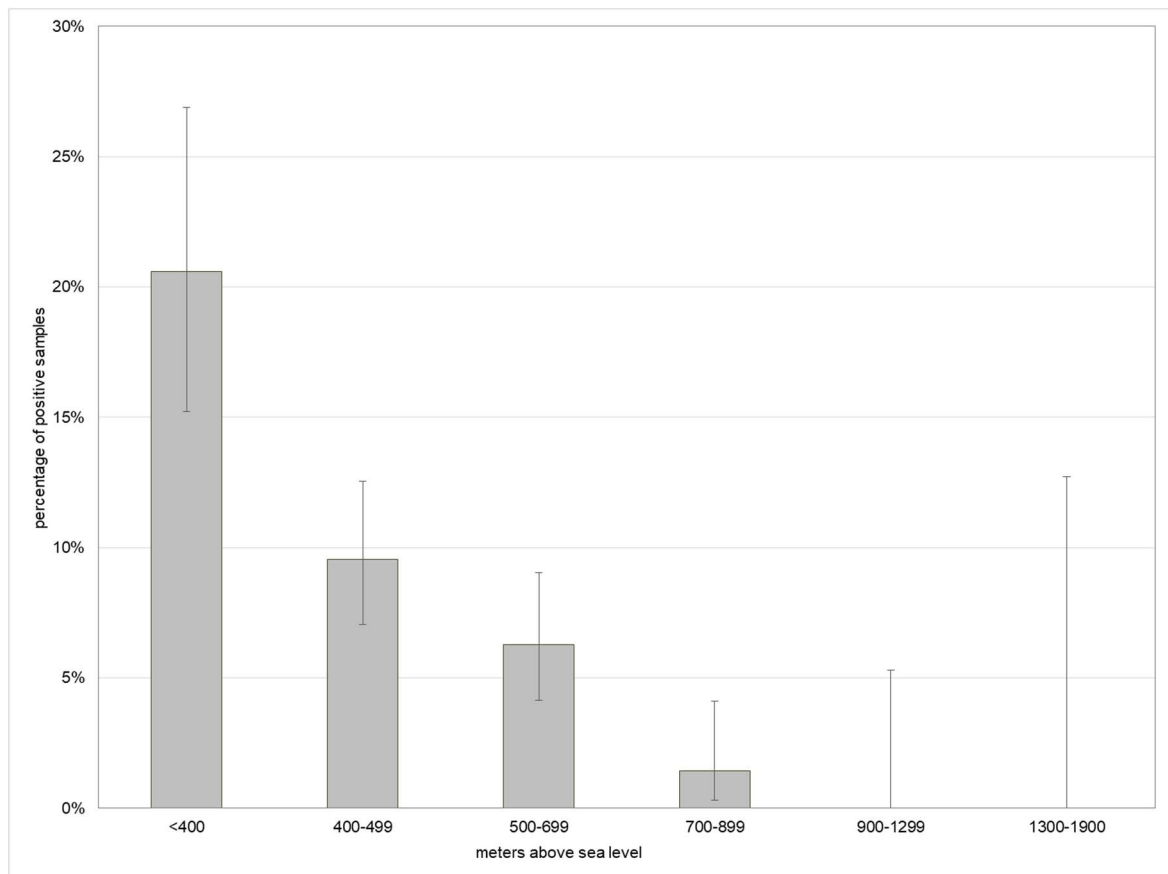
We used an ANCOVA (SPSS 20.0., IBM) to assess environmental factors affecting the presence of the investigated parasites in the faecal samples of the study areas (n=72 areas) with 1) the BIOREGION as a fixed factor and 2) ALTITUDE (mean height above sea level), 3) the percentages of URBANIZED AREA and 4) CULTIVATED LAND and 5) the FAECES FREQUENCY as the covariates. The number of faecal sample per sampling site was used as weighting factor. Exact binomial 95% confidence intervals (CI) for means of binomial variables were calculated according to the method of Clopper and Pearson (1934). Significance levels were set at  $p < 0.05$ .

## Results

A total of 1481 fox faecal samples were collected in the 72 explored areas. Overall, our testing procedures revealed 130 (8.8%, 95% CI: 7.4-10.3%) *A. vasorum* positive samples (distribution: see Fig. 1) all from areas below 900m asl (see Fig. 2), detected by either morphological identification of L1 and/or by genetic analysis.



**Fig. 1:** Sampling areas and occurrence of *Angiostrongylus vasorum* determined by analysis of fox faeces within the four bioregions of Switzerland. Colors and symbol sizes reflect the number of collected samples and parasite frequency (s. legend).



**Fig. 2:** Percentage and 95% Confidence Intervals of fox faecal samples positive for *Angiostrongylus vasorum* collected at different altitudes.

In 7.8% (115/1481) L1 of *A. vasorum* were morphologically identified. Sixty-nine of these samples were tested with the specific *A. vasorum* – PCR and 60 yielded a positive result (87%, CI: 76.7-93.9%). In five out of the remaining nine non-confirmed samples, the broad nematode PCR was positive and sequence analysis confirmed the presence of *A. vasorum* DNA in three samples and the presence of soil nematodes or yeast in another two samples. In none of the samples inhibition was detected. For the lastly remaining four samples the material was not sufficient for genetic confirmation. In summary, genetic analyses were able to confirm the morphological identification in 63/69 samples (91.3%, CI: 82.0-97.7%). In addition, the specific PCR for detection of *A. vasorum* DNA performed with 110 samples containing morphologically undefined larvae revealed 12 further *A. vasorum* positive samples. Supplementary two *A. vasorum* positive samples were identified by PCR when testing 11 samples containing larvae morphologically identified as L1 of *C. vulpis*, indicating a missed mixed infection with the two lungworms, or a morphological misinterpretation, which could not finally be elucidated. Lastly, 19 samples containing larvae morphologically specified as soil nematodes were all negative in the specific *A. vasorum*- PCR, with one exception: a sample containing contemporaneously larvae morphologically defined as L1 of *C. vulpis* and soil nematodes was also PCR-positive for *A. vasorum*.

Further identified helminth stages are listed in Table 1.

**Table 1:** Occurrence of helminths determined in 1481 faecal samples from fox.

Parasite and parasitic stage	N (% and 95% Confidence Intervals)
<i>Angiostrongylus vasorum</i> (L1)	130 (8.8%; 7.4-10.3%)
<i>Crenosoma vulpis</i> (L1)	317 (21.4%; 19.3-21.6%)
<i>Toxocara canis</i> (eggs)	179 (12.1%; 10.5-13.9%)
Taeniidae (eggs)	156 (10.5%; 9.0-12.2%)
<i>Capillaria</i> spp. (eggs)	123 (8.3%; 6.9-9.8%)
<i>Trichuris vulpis</i> (eggs)	82 (5.5%; 4.4-6.8%)
Hookworms (eggs)	79 (5.3%; 4.2-6.6%)
<i>Toxascaris leonina</i> (eggs)	19 (1.3%; 0.8-2.0%)
<i>Strongyloides</i> sp. (eggs)	6 (0.4%; 0.1-0.9%)

The ANCOVA for assessing the effect of the investigated factors (s. Methods) on the parasite occurrence in fox faeces per sampling site (n= 72) revealed significant effects of ALTITUDE, BIOREGION and CULTIVATED LAND (Tab. 2).

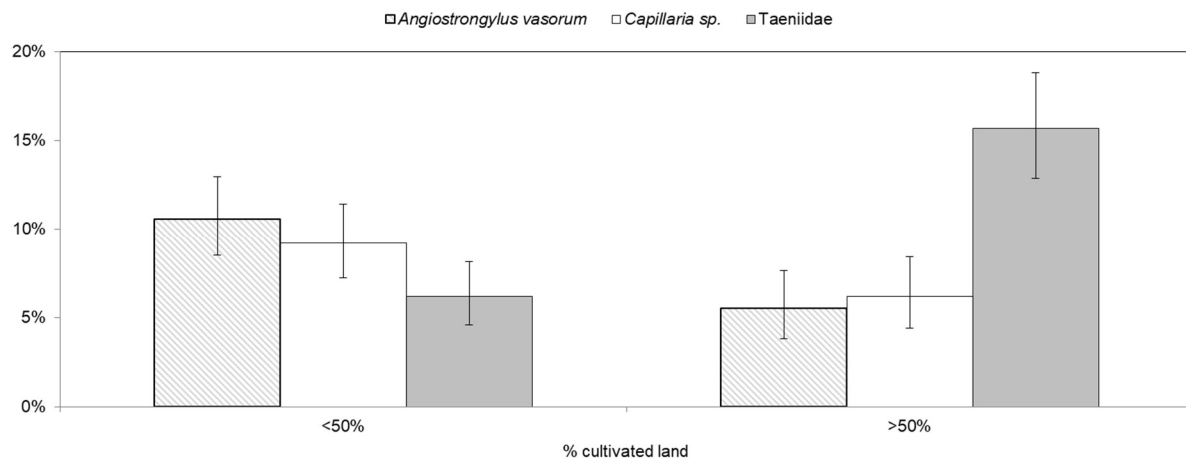
**Table 2:** P-values for the environmental factors, evaluated by ANCOVA, affecting the occurrence of fox helminths as determined by examinations of 1481 faecal samples of foxes (n = 72 localities). In brackets the corresponding F-values are given, significant values ( $p < 0.05$ ) are marked with an asterisk.

Parasite	BIOREGION	ALTITUDE	URBAN AREA	CULTIVATED LAND	FAECES FREQUENCY
<i>Angiostrongylus vasorum</i>	0.018* (3.60)	0.003* (9.76)	0.834 (0.04)	0.043* (4.25)	0.135 (2.3)
Taeniidae	0.113 (2.07)	0.282 (1.18)	0.135 (2.29)	0.010* (7.05)	0.942 (0.01)
<i>Crenosoma vulpis</i>	0.000* (11.68)	0.425 (0.65)	0.704 (0.15)	0.312 (1.04)	0.569 (0.33)
<i>Capillaria</i> spp.	0.007* (4.38)	0.803 (0.06)	0.104 (2.71)	0.049* (4.04)	0.399 (0.72)
<i>Toxocara canis</i>	0.047* (2.80)	0.705 (0.15)	0.871 (0.03)	0.458 (0.56)	0.981 (0)
<i>Trichuris vulpis</i>	0.127 (1.97)	0.953 (0)	0.429 (0.64)	0.18 (1.84)	0.62 (0.25)
Hookworms	0.38 (1.04)	0.867 (0.03)	0.406 (0.7)	0.171 (1.92)	0.72 (0.13)

All these three factors significantly affected the occurrence of *A. vasorum* (BIOREGION:  $F = 3.6$ ,  $p = 0.018$ ; ALTITUDE:  $F = 9.8$ ,  $p = 0.003$ ; CULTIVATED LAND:  $F = 4.2$ ,  $p = 0.043$ ) revealing significantly higher prevalence rates of *A. vasorum* in faecal samples from the Central Plateau (Tab. 3), in regions below 400 m asl (Fig. 2) and in areas with <50% of cultivated land (Fig. 3).

**Table 3:** Occurrence (percentage and 95% Confidence Intervals, CI) of the four helminths for which significant differences were detected between the four bioregions of Switzerland (s. Table 2). Asterisks mark significant differences between the bioregions with the highest (\*<sup>H</sup>) and lowest (\*<sup>L</sup>) prevalences due to non-overlapping confidence intervals (p<0.05).

Parasite	Alps (% , CI)	Central Plateau (% , CI)	Jura (% , CI)	Southern Alps (% , CI)
<i>Angiostrongylus vasorum</i>	1.4 (0.3 - 4.1)* <sup>L</sup>	11.4 (9.4 - 13.8)* <sup>H</sup>	1.6 (0.2 - 5.7)* <sup>L</sup>	6.4 (3.2 - 11.2)
<i>Capillaria</i> spp.	4.7 (2.3 - 8.5)	10.1 (8.1 - 12.3)* <sup>H</sup>	1.6 (0.2 - 5.7)* <sup>L</sup>	5.2 (2.4 - 9.7)
<i>Toxocara canis</i>	10.9 (7.0 - 15.9)* <sup>H</sup>	15.0 (12.7 - 17.6)* <sup>H</sup>	6.4 (2.8 - 12.2)	2.3 (0.6 - 5.8)* <sup>L</sup>
<i>Crenosoma vulpis</i>	10.4 (6.7-15.4)* <sup>L</sup>	28.9 (25.9-32.0)* <sup>H</sup>	4.8 (1.8-10.2)* <sup>L</sup>	0.0 (0.0-1.7)* <sup>L</sup>



**Fig. 3:** Percentage and 95% Confidence Intervals of samples positive for *Angiostrongylus vasorum*, taeniidae and *Capillaria* spp. in sampling sites with less and more than 50% of cultivated land. Percentages for all three helminths differ significantly between areas with less and more than 50% cultivated land (p<0.05, see ANCOVA results of Table 2).

The occurrence of Taeniidae was affected by the percentage of CULTIVATED LAND with higher occurrence of positive samples in areas with > 50% of cultivated land (F=7.0, p=0.010) (Fig. 3), whereas the BIOREGION affected the prevalence rates of *C. vulpis* (F=11.7, p<0.001), with higher prevalence rates in the Central Plateau and lower ones South of the Alps, and of *Toxocara* spp. with lower prevalence rates South of the Alps (F=2.8; p=0.047) (Tab. 3). *Capillaria* spp. were influenced by the BIOREGION (F=4.4, p=0.007; higher prevalence rates in the Central Plateau, Tab. 3) and the percentage of CULTIVATED LAND (F=4.0, p=0.049; higher rates in areas with < than 50% cultivated land, Fig. 3). No significant effect of the



investigated factors could be found for *Trichuris vulpis* and hookworm egg positive samples (Tab. 2).

## Discussion

Our study demonstrated how a sampling scheme for collecting fox faeces, which was developed for ecological studies of foxes (Putman 1984; Sadlier et al. 2004), can be applied for epidemiological investigations on fox parasites. In particular, fox scats findings included the registration of the exact route by GPS which allows the repetition of exactly the same pathway and therefore standardization if repeated over time under the same conditions, without elimination of the animals. Faecal droppings of foxes are frequently placed in visible sites representing territorial marks, but also close to places where foxes were successful in food search; this, together with faecal contents reflecting fox diets, contribute to the correct identification of fox scats (Webbon et al. 2004). To minimize variations, we chose one km<sup>2</sup> areas with alike ecological structure and collected only in autumn and winter, in order to prevent the scats from being exposed to high temperatures. However, a limitation of the study, in contrast to dog data collection, is that the fox scats do not necessarily represent individual animals (unless they are collected from hunted animals, as recently described, Cabanova et al., 2018), as some of the collected samples may have originated from defecations of the same animal(s). This needs to be considered when speaking of “prevalence”. However, with our approach of defining three sampling plots at each site (s. methods), we ensured that our samples originated from different fox families of the investigated study areas.

Our results demonstrate the large-scale occurrence of *A. vasorum* within the Swiss fox population. Serological tests developed for diagnostics in individual dogs and highly suitable for mass-screening lately suggested the broad presence of the parasite in the country, with regional prevalences concerning antibody detection varying from 0 to 7% (Lurati et al. 2015). This latter prevalence was obtained from dogs from the High Rine area, being part of the Central Plateau where interestingly the highest percentage of *A. vasorum* positive fox faeces were detected in this study (11.4%, CI: 9.4-13.8). Towards 90% of the positive samples from dogs originated from areas below 700 m asl (Lurati et al. 2015), while in the here presented study 97.4% of the positive fox samples were collected below 700 m asl, confirming this trend. The prevalence rate was highest at the lowest altitudes (below 400 m asl), while above 900 m asl none was positive. Foxes may be present at altitudes up to 2500m asl (Hausser 1995), while the observed decreasing *A. vasorum* prevalence rates with increasing altitude is suggested to correlate with a lower availability of susceptible snail and slug species that act as intermediate hosts (Boschi 2011; Lurati et al. 2015). In fact, previous studies showed that the higher the fox habitat the less Mollusca are part of fox’ diet (Hartova-Nentvichova et al. 2010). Temperature was furthermore considered relevant for the survival of L1 in the environment before transmission to the intermediate hosts (Ferdushy and Hasan 2010). Accordingly, altitude and cold temperatures were identified as limiting factors for *A. vasorum* occurrence (Jeffery et al. 2004; Lurati et al. 2015).

Besides lower altitudes, the occurrence of *A. vasorum* in fox samples was higher in areas with less than 50% of cultivated land. A recent study investigated the occurrence of *A. vasorum* and *C. vulpis* in dogs from Germany by correlating the postcodes of dog locations with a digital landscape model: interestingly, among 12 differently defined land-use classes, “agricultural field” was identified as a protective factor, meaning that dogs living in such areas were at lower risk of infection with *A. vasorum*. Prevalence differences were attributed to different intermediate host spectra potentially preferring different habitat conditions (Maksimov et al. 2017). In studies performed with 571 frozen faecal samples from red foxes hunted in Slovakia, *A. vasorum* was also detected in areas where temperature reaches up to 25° C in summer and where the average winter temperature drops below -10° C, confirming great climatic adaptability of the parasite (Cabanova et al., 2018). Furthermore, in an univariable regression model of environmental variables, the distribution of none of the examined variables influenced the distribution of *C. vulpis*, while *A. vasorum* was identified to occur by trend in dry areas with

arable land, avoiding humid areas and forests; however, the corresponding interpretations were considered uncertain because of weak relationships (Cabanova et al., 2018). We hypothesise that in cultivated land, in particular in conventionally used agricultural areas where agrochemical products (fertilizers, herbicides and pesticides) are employed, food range and shelter options are reduced for snails and slugs, therefore representing a less suitable habitat. This is supported by a study performed in an area of the Central Plateau of Switzerland (canton Aargau), where the effectiveness of agri-environment schemes (that provide financial incentives for farmers when adopting environmentally friendly agricultural practices (Kleijn et al. 2006)) on biodiversity was evaluated: After five years of implementing ecological compensated areas, species richness of less mobile species such as plants and snails significantly increased (Roth et al. 2008).

For successful endemic establishment of *A. vasorum*, overlapping habitats of intermediate and definitive hosts are required. As foxes (in opposition to dogs) are not subjected to anthelmintic treatments and may feed more frequently on snails and slugs, foxes are believed to play the most important role in *A. vasorum* transmission and establishment in suitable areas and therefore represent a highly useful study subject for evaluating the risk for canine angiostrongylosis. This is supported by constantly higher prevalences of *A. vasorum* in fox populations compared to dog data (reviewed in (Koch and Willeßen 2009)). Possibly, the role of dogs is instead substantial for the fast and larger spread of *A. vasorum* by translocations of infected dogs into new areas: the simultaneous presence of dogs and foxes in areas with access to shared intermediate host (Deplazes et al. 2004; Lurati et al., 2015) possibly facilitates novel establishments of this parasite. The high densities of dogs and foxes in Switzerland can therefore explain the wide-spread establishment and high prevalence rate of *A. vasorum* within a relatively short time span, especially in the densely populated and urbanized Central Plateau. The fox lung worm *C. vulpis* occurs in many regions of the world in bronchi, bronchioles and trachea of wild and domestic canids (Deplazes et al. 2016; Nevarez et al. 2005). Prevalence in foxes may reach up to 87% (Newfoundland, (Jeffery et al. 2004)), but prevalence in Europe mostly ranges between 10 and 25% (Al-Sabi et al. 2014; Rajkovic-Janje et al. 2002; Tolnai et al. 2015), including the here detected prevalence rate of 21.4% (CI: 19.3-21.6%). Its life cycle, like *A. vasorum*, involves snails and slugs as intermediate hosts (Stockdale and Hülland 1970); this constellation was suggested to be the reason behind the clumped and correlated occurrence of both parasites (Taylor et al. 2015; Tolnai et al. 2015). Accordingly, spatial analysis showed a significantly higher occurrence in the Central Plateau, comparable to *A. vasorum*, but contemporaneously a decreased occurrence south of the Alps, as no positive fox sample was identified. First stage larvae of *C. vulpis* were shown to be particularly resistant to harsh cold environmental conditions (Jeffery et al. 2004), supporting findings of higher prevalence in foxes in colder climates of northern Europe (i.e. Germany, 32.3% (Schug et al. 2018)) than in southern Europe (i.e. Italy, 14.7% (Magi et al. 2009)).

Also the frequency of fox faeces with *Capillaria* spp. eggs was significantly higher in the Central Plateau compared to the other bioregions (s. Table 3), and higher in areas with < than 50% cultivated land (s. Fig. 3). Capillariid infections of carnivores are mainly connected with ingestion of earth worms (although there are still open questions regarding other ways of transmission (Deplazes et al., 2016)) which in analogy to snails may be less prevalent in cultivated areas. As no further differentiation was performed, the potential presence of *Capillaria* eggs derived from hunted birds or rodents (intestinal passage) should be also considered.

*Toxocara* egg prevalence in fox faeces (12.1%, CI: 10.5-13.9) was lower compared to several other European fox studies, in which the animals were necropsied and adult worms detected: the percentage of positive animals ranged from approximately 25% up to more than 80% (Borgsteede 1984; Bruzinskaite-Schmidhalter et al. 2012; Saeed et al. 2006; Willingham et al. 1996; Wolfe et al. 2001). Accordingly, in previous studies in dissected foxes i.e. from western

Switzerland and Zurich, a prevalence of 44.3% and 47.4%, respectively, was observed (Hofer et al. 2000; Reperant et al. 2007), indicating higher sensitivity of necropsy for detection of roundworm infections.

The same may account for tape worms: *E. multilocularis* and *Taenia* sp. reached prevalences up to 52.1% and 54.3% in culled foxes from Geneva and up to 66.7% and 16.5% in Zurich, respectively (Hofer et al. 2000; Reperant et al. 2007). Here, taeniid eggs were not differentiated and may therefore include *Taenia* spp. and *Echinococcus multilocularis* as well, but a prevalence of 10.5% (CI: 9.0-12.2%) indicate that the adopted coproscopic methods were far less sensitive for tape worm detection compared to necropsy methods. Interestingly, there was a higher occurrence of positive samples in areas with > 50% of cultivated land. Microtids like *Microtus arvalis* and *Arvicola scherman*, which both are considered as crucial intermediate hosts for *E. multilocularis* (Beerli et al. 2017; Burlet et al. 2011; Guerra et al. 2014), can profit from cultivated land (mainly meadows and pastures), but also other taeniid species (*T. pisiformis*, *T. crassiceps*, *T. polycantha* and *T. serialis*) have rodent intermediate hosts and canid definitive hosts (Deplazes et al. 2016). Overall, although prevalence rates obtained by faecal examination were lower than the ones obtained by necropsy, the large-scale presence of *Toxocara* and of taeniid eggs in the environment support the important role of wild carnivores for transmission to domestic animals and their zoonotic potential (Otranto et al. 2015).

Additional eggs that were identified in the investigated fox samples were, in descending order, eggs of *T. vulpis* (5.5%, CI: 4.4-6.8%), hookworms (5.3%; CI: 4.2-6.6%), *T. leonina* (1.3%; CI: 0.8-2.0%) and *Strongyloides* sp. (0.4%; CI: 0.1-0.9%). They were not significantly associated with any of the investigated factors. For *T. vulpis*, regional studies from Germany or France based on necropsies revealed prevalences up to 10.3% (Petavy et al. 1990; Pilarczyk et al. 2005), while others reported prevalences below 3% (Poglayen et al. 1985; Richards et al. 1995; Vergles Rataj et al. 2013); this places our results as intermediate, taking into account that faecal examination was performed. Ancylostomatids in Swiss foxes are represented by *Uncinaria stenocephala*: adult parasites were identified in a high prevalence (64.8% and 78.2%) in necropsied foxes from Zurich and Geneva, respectively (Hofer et al. 2000; Reperant et al. 2007). In other European countries the prevalence ranged from 26-80% (Al-Sabi et al. 2014; Rajkovic-Janje et al. 2002), also based on necropsies. *T. leonina* may also infect up to 40% of the foxes in other European countries (Al-Sabi et al. 2014; Bruzinskaite-Schmidhalter et al. 2012) including Switzerland (prevalence: 37.3% (Reperant et al. 2007)). *Strongyloides* spp. were instead only rarely reported in foxes; generally, in wild canids they are allocated to *Strongyloides stercoralis*, that is shared not only with dogs, but also with humans and other wild canids (Thamsborg et al. 2017). In the Netherlands faecal examination revealed a prevalence of 0.7% (Borgsteede 1984). In Switzerland, *S. stercoralis* was, to our knowledge, not previously reported in foxes and occurred only rarely in dogs that were mostly imported from Eastern European countries (M. Schnyder, personal communication). In this study no genetic analyses were performed to confirm the findings.

Faecal examination of fox samples delivers indications on spatial trends for the presence of a given parasite on a large-spatial scale without affecting the population under study by culling animals. For lungworms, larval migration methods are recommended for fresh samples only and therefore not feasible with fox faeces collected in the field, while FLOTAC (Schnyder et al. 2010) or the here presented filtration method with larval accumulation can be alternatively used with older samples (Cabanova et al., 2018). This latter method is based on sieving steps and relies on different sizes of the parasitic stages; it was shown suitable for the detection of small taeniid eggs (Deplazes and Eckert 1996) and was therefore supposed to also separate lung worm larvae and eggs from other material, allowing the contemporaneous detection of helminth and cestode eggs and larvae. However, we observed that due to the flotation solution and possibly the age of the samples, the identification of larvae was often challenging, though 96.2% of selected samples morphologically identified as *A. vasorum* L1 were confirmed by

PCR, by which additional positive *A. vasorum* samples were identified that would have been missed with morphological identification only. Limiting factors for successful PCRs are the quantity of available parasite DNA, DNA denaturation or PCR inhibition due to faecal material. In summary, our study confirms that Swiss foxes harbour a wide variety of parasites including zoonotic parasites and parasites representing a risk for the health of the dog population. Among these, *A. vasorum* occurs in all bioregions of Switzerland, but is more prevalent in the Central Plateau, concentrated in regions below 700m asl and less present in areas with a high percentage of cultivated land. The use of the here presented standardized method for faecal collection allows follow-up surveys by collection of fox scats without culling interventions and therefore can be regarded as a useful procedure to assess and monitor over time the infection pressure for dog populations on a large scale.

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**Compliance with Ethical Standards**

This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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